

The Treatment of PHI (Part 1): The Hope of Eradication

Martin H. Markowitz, MD

Clinical Director, Aaron Diamond AIDS Research Center
New York, New York

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AT THE GROUND-BREAKING 1996 INTERNATIONAL CONFERENCE ON AIDS in Vancouver, Drs. Martin Markowitz and David Ho, leading researchers at the Aaron Diamond AIDS Research Center (ADARC), gave presentations charting the gradual depletion of HIV reservoirs in a small group of patients treated with antiretroviral regimens. Dr. Ho went on to suggest that it might be possible to completely eradicate HIV by initiating aggressive antiretroviral, focusing first on a unique population of patients: individuals in the acute stages of HIV infection. The public consideration of what would be, in essence, a cure was met with enormous enthusiasm—and occasional criticism—by the international media, HIV-treating physicians and, of course, people living with HIV.

Needless to say, the original time frame postulated by Dr. Ho and his colleagues—one and a half to three years of “maximally suppressive” antiretroviral therapy—proved to be improbable. Much research generated over the past six years, including several key studies conducted at ADARC, explored two potential caveats that were raised (although rarely reported in mainstream media accounts) by Drs. Ho and Markowitz in 1996: 1) that HIV infection may be widely disseminated during PHI and persist for an extended period of time in certain cellular reservoirs, and 2) that highly active antiretroviral therapy may, in fact, not be potent enough to completely shut down viral replication. Sadly, both warnings proved true, and the hope of eradication all but fizzled out.

“Eradication of HIV may not have been a real expectation, at least in the period of time suggested, given what we now know about the limitations of antiretroviral therapy,” commented Dr. Markowitz. “But this does not mean that we should abandon concepts of eradication or remission. We can talk about starting patients on antiretroviral therapy when their CD4+ cell count is at 500 or at 350. But for some patients, a drop from 500 CD4+ cells to 350 CD4+ cells can occur in a matter of months.

In the long run, regardless of when patients start therapy, they’re still looking at many years of treatment and its various consequences, whether it’s the emergence of short- or long-term side effects, drug-resistant virus, or simply therapy fatigue. Simply put, we really need to determine the best way to use antiretroviral therapy to achieve the greatest amount of benefit in the shortest period of time.”

The Ever-Evolving Eradication Hypothesis

In 1995, experiments conducted at ADARC and the University of Alabama independently established that HIV infection is a highly dynamic process, with a potential to produce more than 10 billion virions daily, even during the asymptomatic stage of disease (Ho, 1995; Wei, 1995). Employing antiretroviral therapy, these teams demonstrated that levels of HIV-RNA in plasma dropped by half every two days or so, indicating that large amounts of viral particles were being produced by infected cells with relatively short life spans. Subsequent experiments documented the existence of a second, slower phase of HIV reduction during therapy, associated with

the loss of replication-competent, long-lived cells in tissue. Mathematical modeling suggested that all cells harboring the virus would die off within about three years of maximally suppressive therapy, thereby raising the possibility of complete eradication of HIV from the human host.

The successful implementation of this model using currently available therapies depended on two key assumptions. First, the model presumed that all of the identified cellular populations had relatively short half-lives. And second, the estimate presumed that the suppression of viral replication achieved by HAART was, in fact, complete suppression of HIV replication.

By November 1997, however, three research reports had laid to rest the first of these assumptions. The reports—representing a series of studies conducted at Johns Hopkins University School of Medicine, the University of California at San Diego, and the National Institutes of Health—confirmed the persistence of a viral reservoir consisting of latently infected, dormant memory CD4+ cells with integrated proviral DNA (Finzi, 1997; Chun, 1997; Wong, 1997). Given estimated half-lives ranging from six to 44 months, eradication would require anywhere from 10 to 60 years of chronic antiretroviral therapy.

Even the more contemporary eradication hypotheses, with their speculations that viral elimination would require decades of continual antiretroviral therapy, are dependent on a key variable—the unconditional halt of viral replication using HAART to completely snuff out latently infected cell populations and to prevent the reseeding of other long-lived cells. Unfortunately, numerous studies completed over the last few years have demonstrated that HIV replication does, in fact, continue in patients who consistently maintain undetectable viral loads as a result of antiretroviral treatment. One set of studies has demonstrated existence of spliced HIV-RNA molecules in cells of patients with prolonged undetectable viral loads, indicating active translation and transcription of viral proteins (Furtado, 1999; Lewin, 1999; Zhang, 1999). A second set of studies found sequence evolution in HIV proteins by phylogenetic analysis, which also suggests ongoing viral replication (Gunthard, 1999; Zhang, 1999). Additional evidence includes collection of CD4+ cells containing unintegrated viral DNA from patients with durable HAART-induced viral suppression—a likely sign of recent cellu-

lar infection (Sharkey, 2000). Another study employing boosted plasma HIV-RNA assays documented cell-free HIV-RNA in blood plasma and genital secretions, another apparent sign of ongoing replication (Dornadula, 1999).

In the face of this new knowledge, which appears daunting at best, investigators at ADARC have been backtracking a bit to understand why HAART is not completely suppressive. It is hoped that by elucidating the reasons for inadequate suppression, HAART could be further optimized, viral replication be completely suppressed, and courses of antiretroviral therapy could perhaps be finite rather than infinite.

Treating Primary HIV Infection: The View From ADARC

If viral eradication or remission is possible, it will likely be documented in patients treated earliest, particularly during primary HIV infection (PHI). The reason for this can be found in the windows of opportunity that have been documented over the past six years of research.

First, if started early enough, HAART may limit the pool size of long-lived HIV-infected cells, such as memory CD4+ cells. Because these cells are rapidly seeded within days of infection, therapy must be initiated promptly and effectively to slow this process. Second, initiating therapy during PHI appears to protect the function of HIV-specific CD4+ cells and, consequently, HIV-specific cytotoxic T-lymphocytes. Protecting the immune response to HIV may translate into a low viral setpoint—which has been shown to translate into long-term clinical benefit—once therapy is halted. This has certainly been the experience of researchers, including Drs. Bruce Walker and Eric Rosenberg, at Massachusetts General Hospital (MGH) in Boston (see “The Treatment of PHI (Part 2): Immune Augmentation Through STIS,” beginning on page 20). Third, HIV is generally quite homogenous immediately after transmission, but the virus then diversifies, limiting the ability of the immune system and antiretroviral treatments to control HIV-RNA levels. This, too, was recently confirmed in a cohort of patients undergoing structured treatment interruptions (STIS) as a part of a PHI treatment protocol at MGH and is also discussed in the *Notebook* article referenced above.

Initiated as a proof-of-concept program

to support their eradication hypothesis back in 1995, Dr. Markowitz and his colleagues at ADARC looked to evaluate the effects of HAART if started within 120 days of PHI. Data were reported on 15 subjects who began treatment, on average, 65 days after the onset of symptoms of PHI. These patients elected to discontinue therapy after 2.6 to 5 years of “apparently suppressive uninterrupted” HAART, durations consistent with time to eradication described in the biphasic decay model.

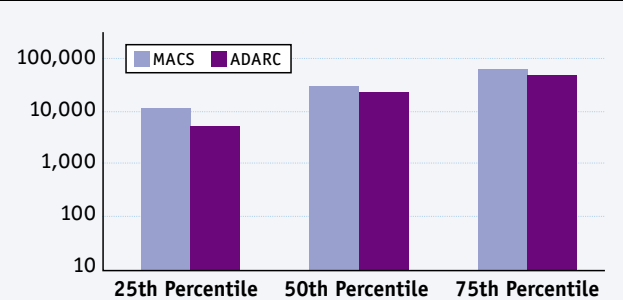
The 15 patients enrolled in this study had relatively more complete seroconversion than those in the MGH cohort described above. At the time therapy was initiated, the ADARC patients had a median baseline viral load of 4.7 log and a pretreatment CD4+ cell count of 542 cells/mm³. Five of the patients received a protease inhibitor-based HAART regimen for their entire treatment period, and ten patients received HAART plus intramuscular injections of ALVAC vCP1452—a recombinant canarypox vaccine with four HIV genes along with an HIV recombinant gp160 component—administered at the start of treatment and then again at days 30, 90, and 180.

At the time of treatment discontinuation, HIV-RNA levels were undetectable (<50 copies/ml) in all except one intermittently nonadherent subject, and the median CD4+ count was 829 cells/mm³. Viral rebounds peaked after a mean of 26 days off treatment. The doubling time was 2.3 days, leading to a mean peak viremia of 4.3 log that spontaneously decreased to a mean nadir of 2.9 log.

Immune studies performed on the day of discontinuation, or within seven days prior, revealed that 7/14 evaluable patients had CD4+ cell proliferative responses (a stimulation index greater than 5) to HIV Gag and 10 of 14 had HIV-specific CTL responses as measured by the number of CD8+ cells expressing interferon-gamma determined by facs after exposure to vaccinia virus expressing Env, Gag, Pol, or Pol-Nef.

Therapy was restarted in 7/15 patients after a mean of 277 days off treatment. At the time therapy was reinitiated in these seven patients, the mean viral load for the entire group had increased to 4.0 log. Three

FIGURE 1. Distribution of HIV-RNA Levels After 1 Year of Untreated HIV Infection (MACS) Versus Last Observed HIV-RNA (ADARC)



Source: Martin Markowitz, MD

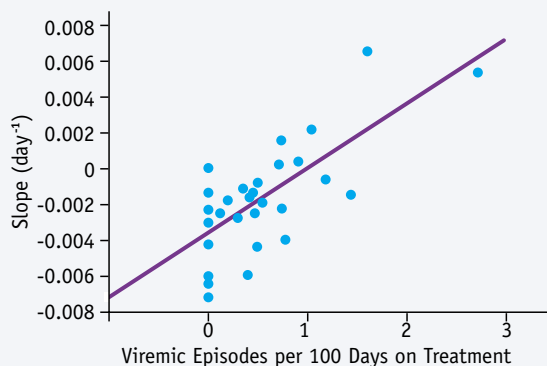
of the original 15 patients have maintained viral loads below 500 copies/mL, in the absence of treatment, for more than 120 days. However, two of the three are heterozygous for CCR5 delta-32, the cellular marker believed to be associated with a more benign HIV course. The five remaining patients all have detectable HIV-RNA levels, in the range of 4 to 5 log, but continue to remain off treatment.

These setpoint levels achieved after treatment was discontinued, when compared with values from patients with documented seroconversion dates in the Multicenter AIDS Cohort Study, showed a similar distribution of HIV-RNA values to individuals who had not received therapeutic intervention during PHI (see Figure 1). In other words, initiating HAART during PHI, either with or without an immune boost provided by the ALVAC therapeutic vaccine, did not appear to alter the natural history of HIV disease once therapy was halted.

What might explain the disparities between the ADARC data and the more encouraging PHI treatment research conducted at MGH? While not specifically discussed by Dr. Markowitz, a few key differences between these two cohorts might account for the differences in the yielded results. First, patients in the MGH cohort were started on treatment sooner than those enrolled in the ADARC studies, suggesting that earlier truly is better when it comes to treating PHI. Second, the ADARC study involved prolonged discontinuation of therapy—regardless of viral load or CD4+ cell count trigger points to restart treatment—whereas the MGH study involved a series of highly controlled STIS.

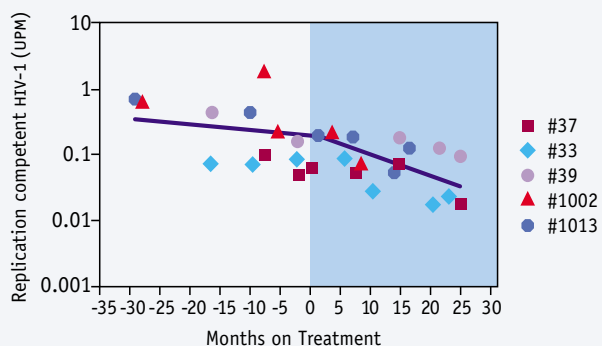
For Dr. Markowitz, the less than encouraging results of the ADARC treatment discontinuation study might be best ex-

FIGURE 2. Correlation Between the Decay Slope of the Latent Reservoir and Episodes of Intermittent Viremia



Source: Bharat Ramratnam, MD

FIGURE 3. Faster Decay of the Latent Reservoir with Treatment Intensification



Source: Bharat Ramratnam, MD

plained by the limits of HAART. “We really need to optimize HAART in order to further reduce residual viral replication,” he stated in his concluding remarks. “HAART also needs to be optimized in order to limit the size of the latent pool and accelerate the decay of latently infected cells.”

Accelerating Decay Rates

If hopes of eradication depend on the brevity of latent reservoirs of HIV infection, then the primary goal of antiretroviral treatment should be to eliminate residual replication and to prevent the occurrence of transient viral load “blips” during therapy. Granted, at least one report in recent years has demonstrated that blips in viral load are not associated with treatment failure and subsequent rebounds in viral load, but it is also clear that intermittent bursts of viremia can essentially undo any decay of latently infected cells

that had occurred prior to the blip (Havli, 2000).

To make better sense of the correlation between the decay of the latently infected reservoir and the extent of ongoing viral replication during HAART, Dr. Markowitz’s colleague Bharat Ramratnam, MD, led a joint-ADARC/Los Alamos National Laboratory endeavor to clarify discrepancies regarding the half-lives of quiescent cells harboring replication-competent HIV. As stated above, estimations of the half-lives of these cells have varied considerably, ranging from six months in one study (Zhang, 1999) to more than 44 months in another (Finzi, 1999). If eradication were to be achieved, Dr. Ramratnam’s team needed to be sure of the actual half-life of the cells in patients with durable viral suppression and to determine the factors that undermine the decay of this cell population.

The group’s findings were reported in the January 2000 issue of *Nature Medicine*. Using a quantitative microculture assay, it was determined that the latent reservoir decayed with a mean half-life of 6.3 months in patients who consistently maintained plasma HIV-RNA levels below 50 copies/mL.

Slower decay rates occurred in individuals who experienced intermittent blips of viremia and were correlated with the number of viremic bursts that occurred throughout therapy (see Figure 2). These findings suggested that the persistence of the latent reservoir of HIV despite prolonged treatment is the result of, not only its slow intrinsic decay characteristics, but also the inability of current drug regimens to completely block HIV replication.

A logical approach to dealing with transient viremia is to intensify the HAART regimen currently being used. In a recent study conducted at ADARC, abacavir (Ziagen), either with or without efavirenz (Sustiva), was added to the regimens of five patients who were experiencing blips in their viral load—defined as intermittent

bouts of HIV-RNA levels above 50 copies/mL—while receiving zidovudine (Retrovir) and lamivudine (Epivir) plus either nelfinavir (Viracept) or ritonavir (Norvir) and saquinavir (Fortovase) for an average of 34 months. After their treatment was intensified, patients were monitored for an additional 14 months. Five matching patients who did not intensify their regimens were used as controls.

To determine if intensification had any effect on the decay rates, the absolute size of the latently infected cell population was determined by analyzing the results of serial-limiting dilution microcultures of highly enriched preparations of CD4+ cells. Co-cultures were expressed as infectious units per million peripheral blood mononuclear cells (IUPM), and linear regression was then used to generate a best-fit line through a plot of IUPM versus time. The slope of this line was then used to calculate the half-life of the latent reservoir.

The mean frequency of viral blips, prior to intensification, was 3.3 per year among the five patients who intensified their treatment and 2.8 per year in the group of control patients. After intensification, the frequency of blips decreased in 4/5 patients, to 0.7 blips per year. In contrast, control subjects remained intermittently viremic; although a decline in the frequency in this group was reported (1.9 per year), this was not statistically significant.

When compared to control patients, the median half-life of the latent reservoir decreased from 31 to 10 months ($P = 0.016$) following intensification; this accelerated decay rate is clearly visible in Figure 3, which includes data from all five patients who underwent treatment intensification. Also reported was a strong inverse correlation between the rate of decay of this latently infected cell population and the frequency of intermittent viremia.

In his summary of these data at a PRN meeting in early 2001, Dr. Ramratnam seemed generally pleased with the hastened decay rates. Unfortunately, better may simply not be good enough. “We definitely saw better viral suppression with intensification in this study,” he commented. “However, we were not able to completely block viral replication, as evidenced by continuing episodes of intermittent viremia. We want to be using highly active therapy, not fairly active antiretroviral treatment.” Well, perhaps we don’t want to use the acronym for that term.


What to Start With: Does Potency Matter?

As demonstrated by the intensification study, HAART as it is currently known appeared to be less than maximally potent. The question was then raised whether more "potent" HAART regimens with novel agents could be designed.

ADARC study 377, headed by Dr. Michael Louie, enrolled 22 patients—11 with PHI and another 11 in the chronic stage of infection—to receive a heady brew of lopinavir/ritonavir (Kaletra); tenofovir DF (Viread), the newly approved nucleotide analogue manufactured by Gilead Sciences; efavirenz, and lamivudine. Data from this study were compared to those of study 197 in which 25 patients, including 12 with PHI and 13 with chronic infection, received a standard dual-class regimen consisting of saquinavir, ritonavir, zidovudine, and lamivudine.

Frequent measurements showed a pharmacologic lag during which viral load did not decrease during the first two days of starting the multiclass regimen. However, HIV-RNA levels subsequently dropped sharply. The first phase of viral decay ended after a mean of 4.1 days, which is much earlier than the 10- to 14-day decay rate determined in the 1995 studies (see above). Similarly, the half-life of productively infected CD4+ cells in this study was 0.7, which is also much shorter than the previous estimate of approximately one day.

The mean change in viral load from day 0 to day 7 was 1.42 log compared to 1.25 for the other four-drug regimen. More important, the decay slope for this phase was $-0.51/\text{day}$ in study 377 compared to $-0.40/\text{day}$ in study 197.

Assuming that the lifespan of infected cells was the same in patients enrolled in both of these studies, the relative potency of the regimens can be determined based on the slopes of the initial viral decline. To do this, the novel four-drug regimen was assigned an arbitrary potency of 1.0; the standard four-drug regimen used in study 197 was determined to have a relative potency of 0.72. The results, Dr. Markowitz pointed out, indicated that the vast majority of antiretroviral drug regimens being used today may be significantly less potent than previously thought. "If eradication is going to be possible," he said, "optimizing HAART will be absolutely necessary." 

KEY POINTS

- Treating HIV during the primary stages of infection may limit the pool size of long-lived HIV-infected cells, such as memory CD4+ cells. Initiating therapy during this window of opportunity might also protect the function of HIV-specific CD4+ cells and, consequently, HIV-specific cytotoxic T-lymphocytes. Early therapy may also help maintain a homogenous viral population that can be better controlled by the immune system and antiretroviral therapy.
- The complete suppression of HIV replication during PHI and chronic infection remains a worthwhile goal, as it may delay the onset of drug resistance, reduce the risk of viremic blips and rebounds, and potentially speed up decay rates and eradication efforts.
- Current antiretroviral therapy, as standardized by the U.S. Department of Health and Human Services, offers only partial suppression of HIV-RNA levels. Efforts to identify regimens that are more completely suppressive and to elucidate their clinical benefits are ongoing.

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